Chapter 3

Liquid Scintillation Counting of Radionuclides Used in Radioimmunoassay

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INTRODUCTION

Many of the radionuclides used in radioimmunoassay- and radioassay-type in vitro diagnostic tests emit gamma rays or X-rays which can be measured in inorganic crystal (NaI) scintillation counters. At the same time there are some radioimmunoassay tests which use tritium as the radioactive label. Tests which use tritium as the label have to use liquid scintillation counting techniques as the means to measure the amount of tritium in the antibody-bound and/or free antigen-separated fractions. It is often not realised that many of the so-called 'gamma and X-ray emitters' upon decay also produce electrons. In some cases the electrons are the result of beta decay while in others they are the results of conversion or Auger electron productions. It is actually possible to measure these electrons by liquid scintillation methods.

Some radionuclides decay by beta emission followed by gamma ray emission. These radionuclides can be measured with high efficiency in a liquid scintillator system depending upon the energy of the beta transition. The radionuclides Iodine-131, Cobalt-60 and Iron-59 decay by the emission of beta particles in 100% of their transitions followed by the emission of one or more gamma ray groups. The maximum energy of the beta emission is such that these radionuclides are measured with high efficiencies (nearly 100%) by liquid scintillation methods. The radionuclides Iodine-125, Cobalt-57 and Chromium-51 all decay 100% by electron capture. As a result they produce emissions of characteristic X-rays and a certain fraction of these events results in the production of Auger electrons. These Auger electrons, because they are mono-energetic, can be counted with high efficiency, depending upon their energy, by liquid scintillation counting methods.

When trying to decide upon measurement of these dual emission radionuclides by either solid inorganic scintillation crystals; gamma and X-rays; or liquid scintillation solutions; beta, conversion electrons and Auger electrons; it should be remembered that any time liquid scintillation methods are used quenching can be a serious problem which will require correction terms applied to the measured results to normalise all data to a common counting efficiency. In measurement of gamma and X-rays with solid inorganic scintillation crystals there are no quenching corrections required.

The advantage of antigen determination by radioimmunoassay and radioassay techniques is primarily the result of the specificity of the antigen-antibody reactions. This property, along with the sensitivity afforded by the use of radionuclides, allows for the measurement of picogramme (10^{-12} g) amounts of a specific antigen without separation of that antigen from all the other antigens present in the serum sample. A single antigen molecule can be labelled with at least one, and often more than one, radioactive atom. The specific activity of the radionuclide will determine the amount of labelled antigen necessary for minimum detection. The short half life of Iodine-125 (60 days) enables one to measure 10,000 c.p.m. from as little as 2×10^{-15} mole of an antigen labelled with one Iodine-125 atom per molecule.

COUNTING OF BETA-GAMMA RADIONUCLIDES

Several radionuclides used as labels for radioimmunoassay and radioassay measurements emit one beta particle per disintegration. Examples of these types of radionuclides are Iodine-131, Iron-59, Cobalt-60 and Sodium-22. Table 1 lists the beta energy groups for each of these radionuclides and their relative abundances. The beta energy groups are all sufficiently energetic to be counted with high efficiency in a liquid scintillator solution.

Table 1. Beta energies and abundance of Iodine-131, Cobalt-60, Iron-59 and Sodium-22 decay modes and their counting efficiencies in a liquid scintillator solution.

Radionuclide	Mode of decay	E_{max} and abundance (%)	Counting efficiency by LSC (%)
Iodine-131	β^-	0.251 (2.8)	95
		0.333 (9.1)	
		0.606 (87.4)	
		0.810 (0.7)	
Cobalt-60	β^-	0.314 (100)	90
Iron-59	β^-	0.130 (0.9)	85
		0.268 (44.9)	
		0.459 (54.9)	
		1.560 (0.3)	
Sodium-22	β^+	0.540 (100)	89

Also included in Table 1 is a typical counting efficiency of each of the radionuclides in a small amount of water (0.05 ml) dissolved in an emulsion-type scintillator solution. As can be seen from this data the counting efficiencies are indeed high and the measurement by liquid scintillation methods appears to be very reasonable. However, the high efficiency still has to be corrected for any quench variation between samples. In all radioimmunoassay and radioassay techniques it is not necessary to know the absolute counting efficiency, but all samples have to be counted with the same efficiency.

Interpretation of the pulse height distributions for samples of these radionuclides in liquid scintillator solutions is fairly simple. Figures 1 (a-d) show the differential pulse height distributions obtained with a liquid scintillation counter which has a logarithmic amplification system (Beckman LS-250 Model). The output of the summing amplifier of the liquid scintillation counter is input directly into the amplifier of a multichannel analyser (Nuclear Data Model 1100) and the coincidence signal from the liquid scintillation counter is used as a gating pulse for the multi-

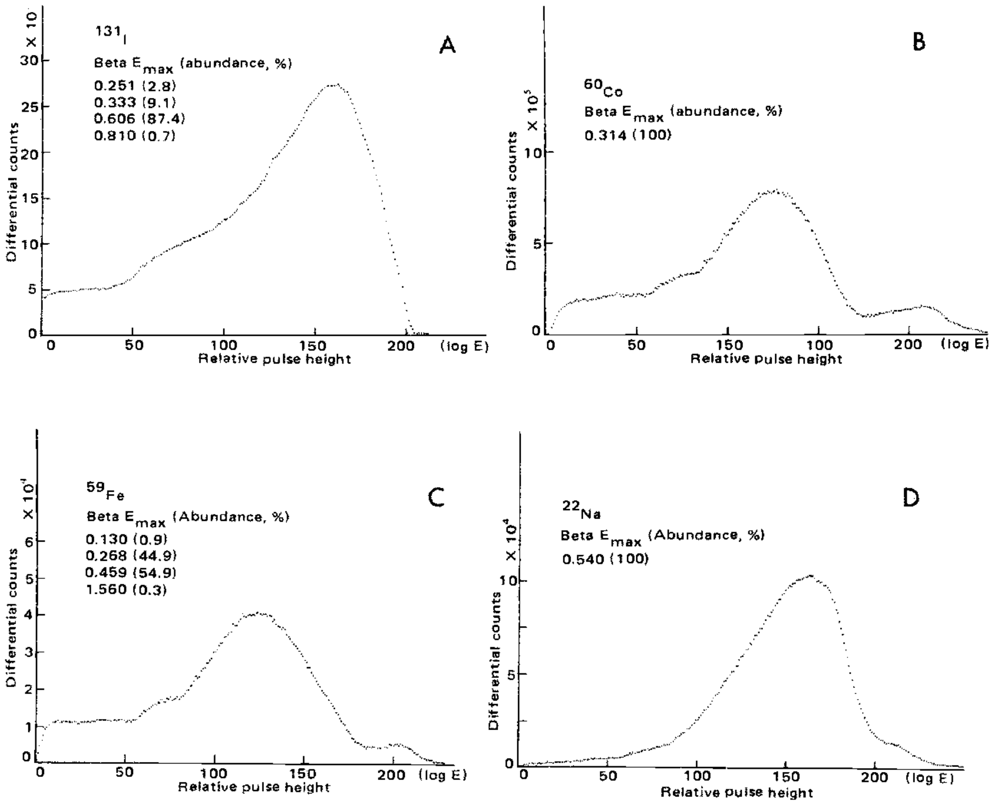

Fig. 1. Differential pulse height distribution for (a) Iodine-131 sample (b) Cobalt-60 sample (c) Iron-59 sample (d) Sodium-22 sample (all aqueous solution) in an emulsifier-containing liquid scintillator solution. Relative pulse height is proportional to the logarithm of the beta particle energy.

channel analyser (after appropriate shaping of the pulse to satisfy the input requirements of the multichannel analyser).

In at least three of the pulse height distributions an additional number of pulses are observed which have pulse amplitudes greater than the E_{max} of the beta spectrum for that radionuclide. These pulses are due to a coincidence between some of the beta particles and Compton scattered electrons produced by the gamma rays which are also emitted by the radionuclide. These can be seen in the pulse height distributions for Iron-59, Sodium-22 and Cobalt-60 but for unknown reasons are not evident in the Iodine-131 pulse height distribution.

COUNTING ELECTRON CAPTURE — AUGER AND CONVERSION ELECTRON RADIONUCLIDES

Table 2 lists the nuclear properties of three other radionuclides used in radioimmunoassay and radioassay tests. These radionuclides all decay by electron capture. The electron capture process will produce either a characteristic X-ray or Auger electron(s). Figures 2 (a-c) show the differential pulse height spectra for Iodine-125, Cobalt-57 and Chromium-51 samples (0.050 ml of aqueous solution in an emulsifier liquid scintillator solution) in a liquid scintillation counter.

Table 2. Liquid scintillation counting of Iodine-125, Cobalt-57 and Chromium-51.

Radionuclide	Mode of decay	Major energy groups (keV)	Counting efficiency by LSC (%)
Iodine-125	Electron capture, conversion electron	12, 40	76
Cobalt-57	Electron capture, conversion electron	14, 136	65
Chromium-51	Electron capture	5.5	30

The Iodine-125 spectrum (Fig. 2a) is composed of two peaks which correspond to the excitations produced by electrons or groups of electrons (Auger electrons and conversion electrons) of energy about 12 keV and 40 keV. These two energy groups are calculated from the decay modes and the probabilities of different processes of energy release. The groups are not due to the stoppage of X-rays in the liquid scintillator solution. Theoretical calculations and experimental measurements both indicate that only about 8% of 27.5 keV X-rays are absorbed in 20 ml of a toluene solution.

The Cobalt-57 spectrum (Fig. 2b) is composed of two peaks which correspond to 14 keV conversion electrons (coincident with low energy Auger electrons) and a somewhat lower intensity 136 keV conversion electron peak. The electron capture of Cobalt-57 produces an excited atom of Iron-57 which decays by the emission of gamma rays, X-rays and conversion electrons. In the liquid scintillator solution only the Auger and conversion electrons are detected.

Figure 2c shows the spectrum for Chromium-51 which is the result of excitations from the 5.5 keV Auger electrons produced by the Vanadium-51 atom which is produced by the electron capture process of Chromium-51. The Chromium-51 process of electron capture occurs 100% of the time. A small contribution to the measured counts comes from the Compton scattering of the 320 keV gamma rays which are produced in 9% of the total decay events.

Table 2 also lists the counting efficiency obtained for samples of these radionuclides dissolved in an aqueous solution and incorporated into an emulsifier containing liquid scintillator solution. The counting efficiency of Iodine-125 is high because nearly every decay event produces a conversion electron or some Auger electrons. Actually the counting efficiency in a 3 in x 3 in NaI(Tl) crystal with a well is also about 75% because nearly every decay event produces either one or two 27.5 keV Te K X-rays.

The counting efficiency of Cobalt-57 is also high because of the high probability of the production of conversion electrons (14 and 136 keV). The counting efficiency for Cobalt-57 in a 3 in x 3 in NaI(Tl) crystal with a well is also high because of the nearly high probability of emission of at least one gamma ray per decay event. These intermediate energy gamma rays (136 and 122 keV) are detected with a high efficiency by the crystal. The counting efficiency in the crystal is about 85%.

The counting efficiency for Chromium-51 in the liquid scintillator solution is low even though 80% of all decay events produce Auger electrons. The low

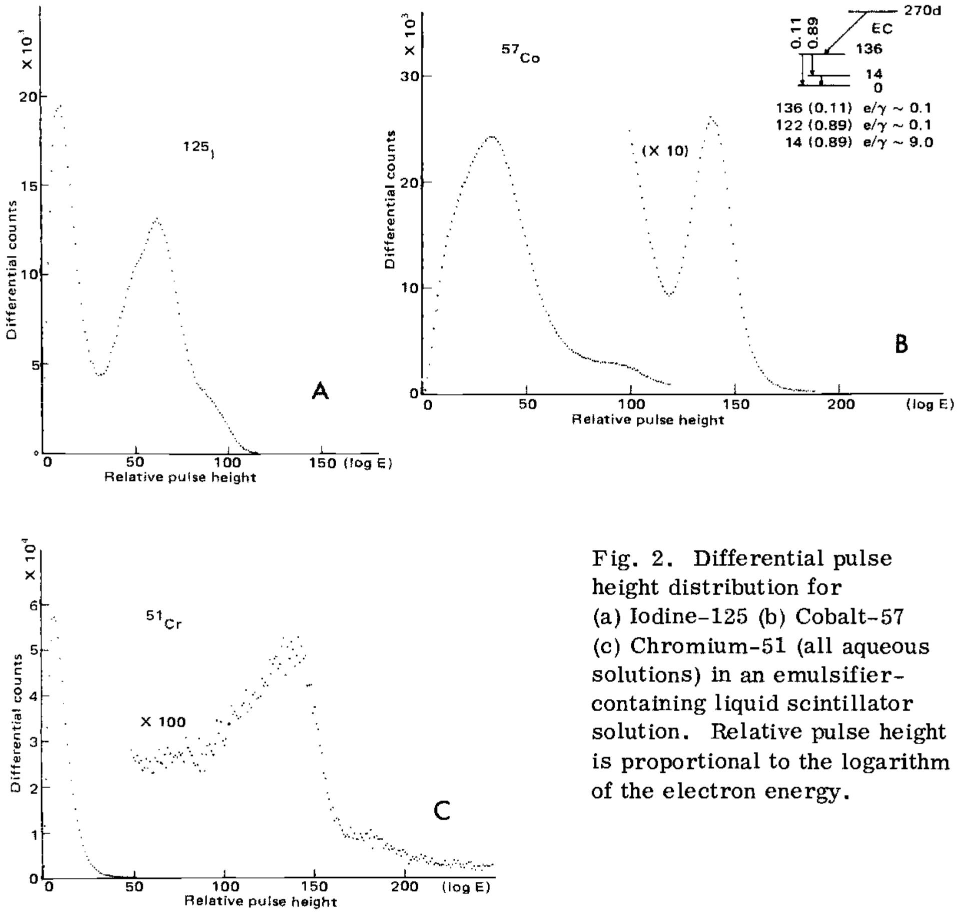

Fig. 2. Differential pulse height distribution for (a) Iodine-125 (b) Cobalt-57 (c) Chromium-51 (all aqueous solutions) in an emulsifier-containing liquid scintillator solution. Relative pulse height is proportional to the logarithm of the electron energy.

efficiency is primarily due to the low energy of these Auger electrons. The most energetic Auger electrons have only 5.5 keV energy. Many of the Auger electrons are less energetic. Even so nearly 40% of the Auger electrons are counted which is reflected in the 30% counting efficiency in the scintillator solution. The counting efficiency is very low in a 3 in x 3 in NaI(Tl) crystal; only about 5.9%. This is primarily due to the fact that only 9% of the total decays produce the 320 keV gamma ray which can be measured in the crystal. Actually this gamma ray is counted with better than 60% efficiency when the abundance is considered. The low energy X-rays from Vanadium-51 are only 5 keV and will not be detected by the crystal. (The low energy X-rays will not penetrate the Aluminium housing of the detector.)

CONCLUSIONS

All these radionuclides can be counted with high efficiencies in a liquid scintillation system. However, there are two main concerns which have to be considered. First, it is necessary to monitor all samples for quench differences between samples with subsequent computation of a quench correction. The second concern involves the possibility of contamination of the liquid scintillation counter with gamma-emitting radionuclides. The high penetration of the electromagnetic radiation would cause an increase in background which would require extensive decontamination before low count rate beta-emitting samples could be measured with any degree of accuracy. Equivalent contamination by beta-emitting radionuclides would be less serious because the beta particles have a low probability of penetration of the bottle walls which is necessary to cause scintillations in the liquid scintillator.

DISCUSSION

J.F.Stoutjesdijk: A difficulty in counting radionuclides emitting γ-rays with a liquid scintillation counter is the influence on the background when samples with greatly different activities are present in the sample changer. A highly active sample adjacent to a low activity sample which is being counted may cause an appreciably higher background. What are the volumes of samples which have been compared in γ-scintillation and liquid scintillation counting? With well type NAI-crystals mostly larger amounts of liquid can be measured, resulting in a higher sensitivity.

D.L.Horrocks: With emulsion-type liquid scintillator solutions it is practical to count up to 3 ml of aqueous samples. Quench correction is possible with external standards and/or sample channels ratio methods.

B.E.Gordon: Why do you prefer emulsion to direct solution in dioxane? Is there any evidence of loss of count rate versus time in dioxane due to adsorption on the walls?

D.L.Horrocks: Emulsion-type scintillation counting solutions are considered less subject to chemiluminescence problems than the dioxane base solutions. Wall adsorptions will be more a function of the samples. However, with emulsions the sample remains in an aqueous environment, and is thus less likely to be salted-out of solution.